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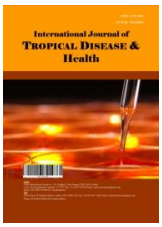

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Evidence of Chikungunya but not Dengue Virus Circulating among Febrile Patients during Low Transmission Period in Morogoro Municipality, Tanzania

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Authors' contributions

This work was carried out in collaboration among all authors. Author LBM wrote the first draft of the manuscript. Authors ESN, LEM and EK managed the literature searches and analyses of the study. All authors read and approved the final manuscript.

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Short Communication

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ABSTRACT

Background: There is currently sufficient evidence available indicating that dengue and chikungunya viruses could be among the causes of fever in Tanzania. Overlapping clinical manifestations of chikungunya and dengue with other vector-borne parasitic diseases pose a challenge for medical diagnosis in Tanzania. A virus surveillance study was conducted in Morogoro Municipality which had no reports of outbreaks during high risk of transmission with dengue epidemics in the neighbouring Dar es Salaam.

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Methodology: The present study was carried out to screen for dengue (DENV) and chikungunya (CHIKV) in sera from patients with fever and malaria-like symptoms on selected health centres in Morogoro municipality (n = 5) during March-May 2018. Three hundred and twelve febrile individuals presenting to the outpatient department were screened for the presence of chikungunya and dengue viruses using Multiplex real-time reverse transcription-polymerase chain reaction.
Results: Acute CHIKV infection was confirmed in four (1.28%) cases whereas no acute DENV infection was detected. Acute chikungunya cases were exclusively prevailing amongst female patients aged between 20 and 49 years.
Conclusion: Our findings indicate an active circulation of chikungunya virus among febrile patients seeking medical attention in Morogoro Municipality, Tanzania. The improvement of CHIKV case detection and reporting is critical to its control and prevention. Surveillance programmes in monitoring arboviral activities in human populations as well as in mosquitos should be performed to avoid maintenance of CHIKV in mosquitoes that may lead to future outbreaks.

Keywords: Chikungunya virus; dengue virus; Tanzania.

1. INTRODUCTION

About 2.5 billion people (40% of the global population) are at risk of dengue infection, while 50-100 million cases of the infection are estimated to occur each year on a global scale [1]. In contrary, chikungunya infection has a very low death rate about <0.1% in hospitalized cases although individuals may experience long term effects such as chronic inflammatory rheumatism which can last up to years after infection [2].

In Sub-Saharan African countries including Tanzania, malaria is prevalent and manifests similar clinical picture with other febrile illnesses [3,4,5]. Thus, misdiagnosis is more probable when these infections occur simultaneously. Recent studies in Tanzania have revealed that patients with acute dengue infection are often misdiagnosed and commonly given presumptive diagnoses of malaria, urinary tract infection and pneumonia [6,7]. These patients are often treated with anti-malaria, antibiotics and anti-fungal drugs [8] which are largely ineffective against dengue and chikungunya. The implication of misdiagnosis and underreporting of arboviral diseases may result in an economic loss [9], development of drug-resistant malaria strains due to over-prescribing of antimalarials and risks of increased morbidity and mortality [10,11].

Several dengue and chikungunya outbreaks have been reported in Tanzania including the recent dengue outbreak in parts of Dar es Salaam (neighbouring region) in 2018 while other studies reported the presence of arboviruses in vector mosquitoes [12,13] and highly suggesting the underlying circulation of arboviruses in Tanzania. Most arboviral studies in Tanzania have been conducted during epidemic periods [1] and little is known about the virus circulation during the inter-epidemic period while more attention is given to the highly endemic regions leaving the non-foci regions at high risk of transmission. Currently, no virus surveillance study has been done to screen for dengue and chikungunya viruses as one of the underlying cause of fever in Morogoro municipality prior a dengue outbreak four years ago in Kilosa District and the recent outbreak in Dar es Salaam that kept this study area at risk of transmission. This study evaluated the occurrence of DENV and CHIKV among febrile patients visiting health centres in Morogoro Municipality.

2. METHODOLOGY

2.1 Study Area

This study was conducted in Morogoro municipality, Tanzania. The municipality occupies a total of 260 square kilometers land coverage with a population of about 315 866 in 2012 and is on increase [14]. The climate of the region is characterized by bimodal rainfall distribution with short rains starting from October to December and long rains from March to May. Sampling was done in urban and peri-urban health facilities that include Mazimbu, Mafiga, Sabasaba, Nunge and Sua health centers (Fig. 1).

2.2 Study Design

A cross-sectional hospital-based study was conducted between March and May 2018, enrolling individuals presenting to the outpatient department with the febrile condition (fever ≥ 38° C). A total of 312 individuals were enrolled based

Fig. 1. Map of Morogoro municipality indicating the five Health centres of this study (indicated by orange colour)

on eligibility criteria and attendance ratio at each of the health centres until the desired sample size was attained. Individuals with chronic and debilitating illnesses were excluded from this study.

2.3 Blood Sample Collection and Processing

Samples from peripheral venous blood (up to 4 ml) were drawn into plain or EDTA anticoagulant tubes at the time of enrollment as per the attached standard operating procedure. Each plain and EDTA tube was labelled with patient biodata. All samples were collected and centrifuged on-site to obtain serum/plasma and furthermore aliquoted into Cryo-vials (2 ml each) and later transported in the cold chain to the laboratory of the National Institute for Medical Research (NIMR) in Morogoro. At the laboratory, these samples were barcoded and stored in freezers at -20°C.

2.4 Laboratory-based Analysis

Stored serum and plasma were subjected to an in vitro automated diagnostic system a one-step multiplex RT-PCR by using AccuPower® RT-PCR kit (Bioneer, Seoul, Republic of South Korea) following manufacturer's instructions. The premix contained specific primers and probe for chikungunya and dengue RNA viruses. It comprises a strip of eight tubes with all components required for cDNA synthesis and PCR, such as M-MLV RT, ribonuclease (RNase) inhibitor, thermostable DNA polymerase, and deoxyribonucleotide triphosphates. The freeze-dried format preserves the viability of the

enzymes for long periods, even beyond typical storage limits.

The premix contained optimal concentrations of all the components necessary for cDNA synthesis and amplification in a single 0.2 ml tube. The primer mix was prepared by mixing 400 nM of forwarding conserved primer (Dcon) and 200 nM of each reversed primer with the appropriate volume of DEPC-treated distilled water. The lyophilized pellet in the tube was dissolved by vortexing, followed by a brief spin. Briefly, 400 µL of NTC (Non-template control) was added into both PC (positive control) and NC (negative control) sample loading tubes, 7µL of PC were added into the PC sample loading tubes and 400 µL of blood serum were added to the remaining sample loading tubes. This includes, 14 blood serum sample loading tubes, 1NTC loading tube and 1PC loading tubes (A total of 16 loading tubes) were placed in one ExiPrep for RNA extraction. A 30 minutes RT-PCR step was performed at 50°C followed by a 15 min Taq polymerase activation at 95°C, 40 cycles of PCR denaturation at 95°C for the 30s, 60°C of annealing for 30s and 72°C extension for 1 min. RT-PCR was performed in a Master cycler gradient machine (Eppendorf, Hamburg, Germany) for 2½ hours. The use of AccuPower RT-PCR premix had greatly facilitated the diagnosis work. Since every single tube contains all the essential components for the RT-PCR premix and fewer steps were required in the protocol which helped to reduce not only potential errors but also the time needed to prepare the assays as well as risks of cross-contamination.

2.5 Data Analysis

Data collected in this study were entered and analyzed using Microsoft Office-Excel 2016 (Microsoft, California, USA) for cleaning and descriptive statistics. The demographic backgrounds including, age, sex, and locality were analyzed. Multiplex real-time RT-PCR data analysis was performed by calculating the threshold cycle (CT) value represented by the positive amplification of the gene in the RT cycle numeral.

3. RESULTS

3.1 Demographic Profiles of the Enrolled Outpatients

There were 312 febrile individuals enrolled in the study whereas a high number of participants were young adults 125 (40%) aged between 20 and 29 years. The majority of enrollments were from Mazimbu hospital (42%) followed by Nunge (21%), Mafiga (14%), Sabasaba (12%) and SUA (12%) health centres, respectively. Out of these study participants, 230 (74%) were females and 82 (26%) were males (Table 1).

3.2 Screening of Dengue and Chikungunya Viruses

Out of 312 serum and plasma samples tested by multiplex real-time RT-PCR, only four (4) febrile

Table 1. Prevalence of DENV AND CHIKV infections among febrile outpatients and their social demographic characteristics between March and May 2018, in Morogoro municipality, Tanzania

Variables	Level	Frequency n (col %)	DENV % (n^{α} /N)	CHIKV % (n^{α}/N)
Gender	Male	82 (26%)	-	_
	Female	230 (74%)	-	1.7% (4/230)
Age (in years)	0-9	17 (6%)	-	-
	10 -19	48 (15%)	-	-
	20-29	125 (40%)	-	1.6% (2/125)
	30-39	60 (19%)	-	1.7% (1/60)
	40-49	29 (9%)	-	3.4% (1/29)
	>50	33 (11%)	-	-
Hospital/Health care centres	Mazimbu	130 (42%)	-	1.5% (2/130)
	Mafiga	43 (14%)	-	-
	Nunge	64 (21%)	-	1.6% (1/64)
	Sabasaba	38 (12%)	-	-
	SUA	37 (12%)	-	2.7% (1/37)

** n= number of participants; n^{α}= number of positive patients; N= total number of participants tested in each variable level; SUA – Sokoine University of Agriculture*

patients (1.28%) confirmed acute CHIKV infection. However, none of the 312 participants had confirmed acute DENV infection (Fig. 2). Dengue and chikungunya cases were classified according to WHO [15] criteria. Thus, the acute chikungunya case definition was confirmed by positive PCR results. Chikungunya cases were exclusively prevailing amongst females febrile patients aged between 20 and 49 years (Table 1).

4. DISCUSSION

Monitoring arbovirus infections as part of virological surveillance in humans including virus isolation from human serum and mosquito inoculations has greatly influenced outbreak predictions globally [16]. The study has demonstrated the presence of CHIKV infection in febrile patients seeking medical care in health facilities during the non-epidemic period. In malaria-endemic areas, most febrile illnesses have been and are treated as malaria cases due to lack of diagnostic capacity [3,4,10,15]. This is attributed by the similar clinical picture in the initial stage of illness of dengue, chikungunya and other febrile illnesses. The diagnostic capacity to rule-out chikungunya, dengue and other infections has remained an obstacle to their control and prevention in resource-limited settings [12].

This study has revealed 1.3% of CHIKV occurrence and suggesting active CHIKV transmission in Morogoro Municipal. This prevalence is less than the previously reported chikungunya prevalence in different regions of the country [6,17,18,19]. The variation in many studies may be endorsed by the time factor, seasonal variations and climatic changes [8]. Furthermore, CHIKV infection symptoms last for about 2-10 days, the time in which virus is

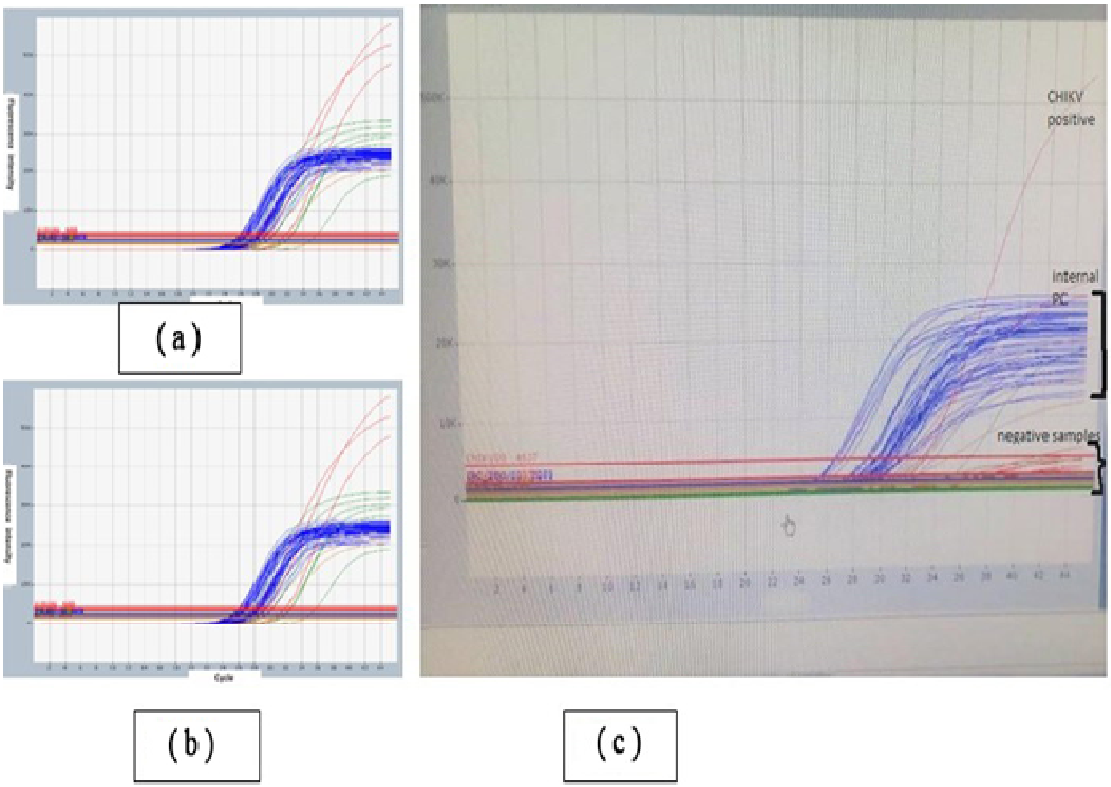

Fig. 2. a; The amplification plot of multiplex real-time RT-PCR results, where each colour represents a specific virus, Green- DENV, Blue-IPC, Red- CHIKV, and Brown- ZIKV. b; the amplification plot of Multiplex real-time RT-PCR showing the internal positive control and (CHIKV & DENV) negative samples. c; The amplification plot of Multiplex Real-time RT-PCR of positive CHIKV showing the internal positive control and CHIKV positive samples as the CT values indicated in Table 2

Table 2. Ct values for positive chikungunya virus samples

Sample ID	CHIKV CT	DENV CT
IPC	28.49	28.14
29	27.2	-
128	32.01	-
146	35.08	-
197	30.04	-

present in the blood circulation and it can be detected by PCR only within this limit. The sensitivity of acute infection diagnosis after the viremic phase becomes minimal as virus levels are reduced significantly [18].

In this study, chikungunya cases were exclusively present in adult females ranging from 20-49 years of age. Similarly, other studies reported adulthoods as a potential risk factor to DENV and CHIKV infections [5,20].

These findings differ from a study conducted in Magugu district [17] which reported that male sex had five times higher odds of being CHIKV positive. Other studies have demonstrated that age and sex are proxy behaviour that causes higher exposure to mosquito bites [21,22]. Most adults aged between 18 and 55 years practices a lifestyle of staying outdoors during dusk and dawn hours which favours *Aedes* mosquito bites. Consequently, such exposure to mosquito bites propensity makes them vulnerable to mosquito-borne diseases including dengue and chikungunya [23]. However, cultural dynamics in Tanzania where females are mostly responsible for farming, housekeeping, cooking, fetching water and firewoods, among other risk factors such as reduced body immunity in lactating and pregnant mother, these exacerbate exposure and development of the disease. Also, employed individuals and livestock keepers who are associated with daily movements from home to work have an increased chance of contracting *Aedes* mosquito bites at early morning and dusk from different locations [24].

This study has shown that there were no DENV cases at the selected health centres despite its serological evidence in Morogoro [6] and other regions of the country [17,18,21]. Most of dengue and chikungunya studies reported in Tanzania were conducted during an outbreak.

The absence of DENV in the collected samples could be due to the low rate of DENV circulation and perhaps, due to sampling in the non-epidemic period.

5. CONCLUSION

Febrile patients visiting health centres in Tanzania are routinely misdiagnosed and treated as malaria infection. Our findings show that CHIKV is an important cause of fever during the inter-epidemic period. Screening and engrossment of a differential diagnosis for arboviral infections and neglected tropical diseases is crucial to improve case management and public health.

CONSENT

All authors declare that written informed consent was obtained from the patient for publication of this report and accompanying images.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed by the ethical standards laid down in the 1964 Declaration of Helsinki**.** Ethical approval was obtained from the Medical Research Coordinating Committee (MRCC) of the Tanzania National Institute for Medical Research (NIMR/HQ/R.8a/Vol.IX/1896) and permission was sought from the Morogoro Region Health Authority to conduct the study in the health facilities.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. WHO. Fourth WHO Report on neglected tropical diseases: Integrating neglected

tropical diseases into global health and development. IV WHO Report on Neglected Tropical Diseases; 2017.
Available:https://doi.org/WHO/HTM/NTD/2017.01

2. Bloch D. The cost and burden of chikungunya in the Americas. New Haven EliScholar, USA: Yale University; 2016.
3. Kinimi E, Patrick BN, Misinzo G. Serological evidence of chikungunya and malaria co-infection among febrile patients seeking health care in Karagwe district, Tanzania. Tanzania Journal of Health Research. 2018a;20(4).
4. Mishra B, Sharma M, Pujhari SK, Ratho RK, Gopal DVRS, Kumar CN, Varma SC. Utility of multiplex reverse transcriptase-polymerase chain reaction for diagnosis and serotypic characterization of dengue and chikungunya viruses in clinical samples. Diagnostic Microbiology and Infectious Diseases. 2011;71(2):118–125.
5. Ndosi R, Kwigizile E, Ibrahim U, Dossajee U, Rwiza J, Kabanyana C, Chilongola J. Risk factors for concurrent malaria and arbovirus infections in Handeni, Northeastern Tanzania. International Journal of Tropical Disease & Health. 2016;20(4):1–7.
6. Chipwaza B, Mugasa JP, Selemani M, Amuri M, Mosha F, Ngatunga SD, Gwakisa PS. Dengue and chikungunya fever among viral diseases in outpatient febrile children in Kilosa District Hospital, Tanzania. PLoS Neglected Tropical Diseases. 2014;8(11).
7. Londhey V, Agrawal S, Vaidya N, Kini S, Shastri JS, Sunil S. Dengue and chikungunya virus co-infection: The inside story. Journal of Association of Physicians of India. 2016;64(3):36–40.
8. Hertz JT, Munishi OM, Ooi EE, Howe S, Lim WY, Chow A, Crump JA. Chikungunya and dengue fever among hospitalized febrile patients in Northern Tanzania. American Journal of Tropical Medicine and Hygiene. 2012;86(1):171–177.
9. Hume JCC, Barnish G, Mangal T, Armázio L, Streat E, Bates I. Household cost of malaria overdiagnosis in rural Mozambique. Malaria Journal. 2008;7(33): 1475-2875.
10. D'Acremont V, Kilowoko M, Kyungu E, Philipina S, Sangu W, Kahama-Maro J, Genton B. Beyond malaria--causes of fever in outpatient Tanzanian children. The New England Journal of Medicine. 2014;370(9):809–817.
11. Oladosu OO, Oyibo WA. Over diagnosis and overtreatment of Malaria in children that presented with fever in Lagos, Nigeria. ISRN Infectious Diseases. 2013;914675.
12. Mboera LEG, Mweya CN, Rumisha SF, Tungu PK, Stanley G, Makange MR, Oriyo NM. The risk of dengue virus transmission in Dar es Salaam, Tanzania during an Epidemic Period of 2014. PLoS Neglected Tropical Diseases. 2016;10(1):1–15.
13. Mweya CN, Kimera SI, Stanley G, Misinzo G, Mboera L. Climate change influences potential distribution of infected Aedes aegypti Co-Occurrence with Dengue Epidemics Risk Areas in Tanzania. Plos One. 2016;11:e0162649.
14. Tanzania Nation Bureau of Statistics; 2017.
Available: http://www.nbs.go.tz/
15. WHO. Regional Office for South-East Asia. Dengue: Guidelines for diagnosis, treatment, prevention, and control. Special Programme for Research and Training in Tropical Diseases; 2009.
Available:https://doi.org/WHO/HTM/NTD/DEN/2009
16. Gubler DJ. Dengue and dengue hemorrhagic fever dengue. Clin Microbiol Rev. 1998;11(3):480-496.
17. Faustine NL, Sabuni EJ, Ndaro AJ, Paul E, Chilongola JO. Chikungunya, dengue and west nile virus infections in Northern Tanzania. JAMMR. 2017;24(4):1–7.
18. Kajeguka DC, Kaaya RD, Mwakalinga S, Ndossi R, Ndaro A, Chilongola JO, Alifrangis M. Prevalence of dengue and chikungunya virus infections in north-eastern Tanzania : A cross-sectional study among participants presenting with malaria-like symptoms. BMC Infectious Diseases. 2016;16(183):1-9.
19. Kinimi E, Shayo MJ, Patrick BN, Angwenyi SO, Kasanga CJ, Weyer J, Misinzo G. Evidence of chikungunya virus infection among febrile patients seeking healthcare in selected districts of Tanzania. Infection Ecology and Epidemiology. 2018b;8(1): 1553460.
20. Mardekian SK, Roberts AL. Diagnostic options and challenges for dengue and chikungunya viruses. BioMed Research International. 2015;834371.
21. Bisimwa NP, Angwenyi S, Kinimi E, Shayo M, Bwihangane BA, Kasanga CJ. Molecular detection of arboviruses in

Aedes mosquitoes collected from Kyela district, Tanzania. Revue de Medicine Veterinaire. 2016;167(5–6).

22. Furuya-Kanamori L, Liang S, Milinovich G, Soares Magalhaes RJ, Clements ACA, Hu W, Yakob L. Co-distribution and co-infection of chikungunya and dengue viruses. BMC Infectious Diseases. 2016; 16(1):1417-2.
23. Dhimal M, Gautam I, Joshi HD, O'Hara RB, Ahrens B, Kuch U. Risk factors for the presence of Chikungunya and dengue vectors (*Aedes aegypti and Aedes albopictus*), Their altitudinal distribution and climatic determinants of their abundance in central Nepal. PLoS Neglected Tropical Diseases. 2015;9(3).
24. Camara N, Ngasala B, Leyna G, Abade A, Rumisha S, Ndekya M, Mboera L. Socio-demographic determinants of dengue infection during an outbreak in Dar es Salaam City, Tanzania. Tanzania Journal of Health Research. 2018;2(20): 1-7.

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